

# Functional Sperm of the Yellowtail (*Seriola quinqueradiata*) Were Produced in the Small-Bodied Surrogate, Jack Mackerel (*Trachurus japonicus*).

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journal or publication title	Marine Biotechnology
volume	17
number	5
page range	644-654
year	2015-10
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科学研究費研究課題	サケ科魚類の進化に伴うGSC制御機構の変化 Evolution of GSC regulation in salmonids Planned
研究課題番号	25114005
URL	<a href="http://id.nii.ac.jp/1342/00001607/">http://id.nii.ac.jp/1342/00001607/</a>

doi: 10.1007/s10126-015-9657-5



1 **Figure legends**

2 **Fig. 1** Section of the donor testis of the yellowtail stained with hematoxylin and eosin. **a** Overview of  
3 the testis. Upper and lower rectangles show the area rich in type A spermatogonia and sperm,  
4 respectively. **b** and **c** show higher magnification images of upper and lower rectangles in **a**, respectively.  
5 Scale bars: 2 mm (**a**) and 20  $\mu$ m (**b** and **c**).

6

7 **Fig. 2** Intraperitoneal transplantation of donor testicular cells into the jack mackerel larvae. **a**  
8 Bright-field image. **b** Fluorescent image. **c** Transplanted (TP) and non-transplanted (NTP) individuals.  
9 N, transplantation needle. Scale bar = 1 mm.

10

11 **Fig. 3** Incorporation of transplanted PKH26-labeled cells into recipient genital ridges. Fluorescent (**a**,  
12 **c**) and yellowtail *vasa in situ* hybridization (**b**, **d**) images of the excised genital ridges from  
13 transplanted (**a**, **b**) and nontransplanted (**c**, **d**) fish at 20 dpt. *Arrowheads* indicate donor-derived germ  
14 cells that were labeled with PKH-26 (**a**), and were expressing the yellowtail *vasa* mRNA (**b**). Scale bars  
15 = 20  $\mu$ m.

16

17 **Fig. 4** Sections of jack mackerel (**a**, **b**) and the yellowtail (**c**, **d**) testis hybridized with antisense jack  
18 mackerel (**a**, **c**) and yellowtail *vasa* probes (**b**, **d**). Scale bars = 20  $\mu$ m.

19

20 **Fig. 5** RT-PCR amplification of donor-derived yellowtail *vasa* mRNA in recipient gonads.

21 Electrophoresis patterns of RT-PCR of recipient number 1–14 (**a**) and 15–28 (**b**) using specific primers  
22 for the yellowtail *vasa* (upper panels) and jack mackerel *vasa* (lower panels) cDNA sequences. *Lane P*  
23 is a positive control obtained using cDNA derived from the yellowtail testis as template. *Lane N* is a  
24 negative control containing no cDNA template. *M* represents a molecular weight marker.

25

26 **Fig. 6** Detection of the colony containing donor-derived germ cells in recipient testis. Serial sections of  
27 testis from jack mackerel testis hybridized with yellowtail (**a**) and jack mackerel (**b**) *vasa* probes and  
28 were stained with hematoxylin and eosin (**c**). The colony containing donor-derived germ cells is  
29 surrounded with a dotted line. Scale bars = 20  $\mu$ m.

30

31 **Fig. 7** PCR amplification of donor-derived yellowtail *vasa* genomic DNA fragment in recipient semen.  
32 *Lane P* is a positive control obtained using genomic DNA extracted from the yellowtail semen as  
33 template. *Lane N* is a negative control containing no DNA template. Electrophoretic mobility of the  
34 targeted DNA fragment was indicated by *arrowheads*.

35

36 **Fig. 8** Embryos at 48 h-post-fertilization obtained from progeny tests. **a** Normal embryos obtained by  
37 crossing the yellowtail female and male. **b** Eggs obtained by crossing the yellowtail female and male  
38 recipient number 28. *Arrowhead* indicates a normal embryo. **c** Eggs obtained by crossing the yellowtail  
39 female and wild-type jack mackerel male. Scale bars = 1 mm.

40

41 **Fig. 9** Donor-derived offspring obtained from progeny tests. **a** Newly hatched larva obtained by  
42 crossing the yellowtail female and male. **b–d** Newly hatched larvae obtained by crossing the yellowtail  
43 female and male recipient number 28. **b** Normal individual. **c, d** Abnormal individuals. Scale bars =  
44 500  $\mu\text{m}$ .

45

46 **Fig. 10** PCR amplification of  *$\beta$ -actin* and *vasa* genomic DNA fragments in larvae obtained from  
47 progeny tests. Electrophoresis patterns of PCR using specific primers for yellowtail  *$\beta$ -actin* (**a**), jack  
48 mackerel  *$\beta$ -actin* (**b**), yellowtail *vasa* (**c**), and jack mackerel *vasa* (**d**) DNA sequences. *Lane 1* and *2* are  
49 larva samples obtained from the crosses of recipient mackerel number 28 and the yellowtail females.  
50 *Lane 3* and *4* are larva samples of wild type yellowtail. *Lane 5* and *6* are wild type jack mackerel. *Lane*  
51 *7* is a negative control containing no DNA template. *M* represents a molecular weight marker.

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